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Stem Cell Plasticity?

Minireview

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Cells differentiate according to stereotype pedigrees, or at least so we thought. Several studies have challenged this dogma and suggested that stem cells in several tissues may be plastic and switch lineages, but many of the results are open to other interpretations. Is there solid evidence for stem cell plasticity and should we rewrite the textbooks just yet?

Distinct lineages emerge from pluripotent cells during the succession of early embryogenesis, and progressively more restricted cells give rise to the specialized cells of different organs and tissues. Decades of developmental studies have provided us with a family tree for the generation of the major classes of cells in the body, which has unveiled robust, stereotype pedigrees. Cells have been thought to only progress in one direction along these differentiation pathways and to be unable to switch tracks. In many tissues, self-renewing multipotent stem cells are maintained in adulthood and serve to replace cells that have a limited life span or to regenerate cells after injury. Such stem cells were believed to be restricted in their potential and limited to generate the types of cells present in the tissue in which the stem cell resides. For example, a neural stem cell would be restricted to generate neural cells and an epidermal stem cell to make skin cells.

A flurry of studies over the last few years has challenged this concept, suggesting that certain tissue stem cells in embryos and adults may be more plastic than previously thought and may give rise to cells of unrelated lineages if transferred to another environment. In this new environment, the stem cell would be able to respond to the novel instructive cues, which would reprogram the cell to generate cells appropriate for the new environment. This concept is known as stem cell plasticity.

Several recent studies have, however, suggested alternative explanations to some of the findings which have been ascribed to stem cell plasticity, and questioned the existence of this event. When studying these processes, there are numerous caveats that pose a serious risk of erroneously interpreting findings as signs of stem cell plasticity.

It is important to unravel the potential extent and molecular biology of stem cell plasticity for several reasons. First, this concept challenges our view of how cellular differentiation is regulated. Second, it poses the question of whether this process may be in effect during normal physiological conditions and in pathological situations. Third, stem cells may offer an attractive source of cells for transplantation, and the concept of stem cell plasticity implies that certain adult stem cells may be much more potent and versatile than previously thought. In the ultimate situation, they could offer an ethically uncontroversial and autologous alternative to embryonic stem cells in therapeutic situations.

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Lineage Infidelity—from Fruit Fly to Man

A burst of papers in the last few years has suggested stem cell plasticity in different experimental situations in mice. The common theme in these studies has been to follow the fate of genetically marked cells in a new environment and analyze whether cells of other lineages are generated. Among the more striking examples are reconstitution of hematopoiesis in irradiated mice by intraveneous injection of neural stem cells, contribution of neural stem cells to tissues of all three germ layers when injected into early chick or mouse embryos, and the generation of, for example, liver cells, myocytes, and even neurons from bone marrow cells (Ferrari et al., 1998; Bjornson et al., 1999; Clarke et al., 2000; Lagasse et al., 2000; Brazelton et al., 2000; Mezey et al., 2000; Krause et al., 2001).

Although most of the recent studies suggesting stem cell plasticity have been performed in mice, there are examples suggesting similar phenomena in both *Drosophila* and man. In *Drosophila*, specialized appendages such as legs and wings are formed from clusters of undifferentiated cells called imaginal discs. When cells are transplanted between imaginal discs, most transplanted cells retain their positional identity, but some transplanted cells will acquire the positional identity of the new location, a phenomenon known as transdetermination (Maves and Schubinger, 1999). There are several findings indicative of stem cell plasticity in humans. By tracking cells of male origin carrying a Y chromosome in patients who have received transplants from a donor of opposite sex, it has been possible to track the progeny of grafted cells. Several studies have demonstrated XY liver cells in women receiving a transplant of male hematopoietic stem cells or bone marrow, and XY cells in the liver of males which have received a liver from a female donor, suggesting that blood and bone marrow derived stem cells can generate hepatocytes in humans (Alison et al., 2000; Theise et al., 2000).

One can envision three conceptually different ways for a cell to switch lineages (Figure 1). Transdifferentiation is the situation where a fully differentiated cell takes on another differentiated phenotype, often without cell division. Alternatively, the lineage switch can be executed by transdetermination. Here, a stem or progenitor cell which is determined to generate a specific set of cell types switches properties to that of another stem or progenitor cell determined to generate another set of differentiated cells. Third, a cell can switch lineage by first dedifferentiating to a common stem or progenitor cell and then redifferentiating to another distinct cell type. Importantly, in no study suggesting stem cell plasticity in mammals has data been provided indicating in which of the above outlined ways a certain cell may have switched lineage.

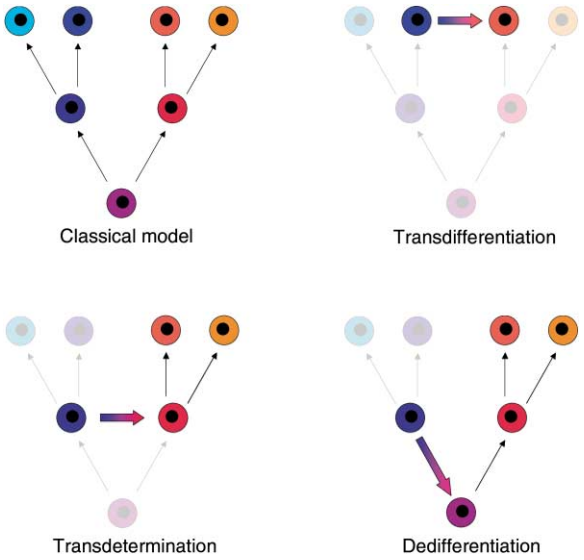

Figure 1. Multiple Paths to a New Identity

In the classical model, cells differentiate by strict progression along different branches of a linear pedigree. In this image, a common stem cell (at the bottom of the family tree) generates two stem cells which are determined to generate two different specialized cell types.

Three conceptually different ways how a cell can switch lineage are depicted. A differentiated cell can take on the phenotype of another differentiated cell, known as transdifferentiation. Examples of this include the generation of a lens from retinal pigment cells after eye injury in newts and the transdifferentiation of smooth muscle cells to skeletal myocytes in the esophagus during normal mammalian development (Patapoutian et al., 1995).

Transdetermination is the situation in which a stem cell that is determined to generate cells of a certain lineage switches to another stem cell state and generates progeny of the latter lineage. This can be seen when cells are transplanted between imaginal discs in *Drosophila* larvae.

Dedifferentiation to a common, more potent stem cell, followed by differentiation along another lineage, is an alternative way for a cell to switch lineage. The cell which dedifferentiates could be a fully differentiated cell, or as in this image, a determined stem cell. Dedifferentiation is seen for example after limb amputation in newts, which results in dedifferentiation of local myocytes, followed by regeneration of cells of different lineages.

Phenomenology, Pathology, or Physiology?

It is important to underscore that many of the experimental situations where stem cell plasticity has been implied are artificial and may be far from reflecting a physiological situation. Some may resemble situations of pathology, whereas others do not resemble situations seen in real life but are purely experimental, such as for example injecting adult cells into embryos. Moreover, in some experiments, stem cells are cultured before being exposed to a new environment, which may make them lose their positional bearings and be more amenable for switching lineage. It is hence important, as phrased in a recent review, to distinguish between the actual and the possible (Anderson, 2001). Although some of the experimental situations are far from what is seen in physiological situations, it may be informative to study how, for example, cellular differentiation and determination are influenced in extreme situations. This is underscored in recent studies of cellular reprogramming by nuclear transfer, an artificial situation which promises to teach us much about fundamental aspects of cellular determination.

In many situations where stem cell plasticity has been implied, there has been damage to the tissue where the new cells of unrelated lineage emerge, and there are some indications that this may be a prerequisite for the observed effects. For example, extremely few hematopoietic stem cell-derived liver cells are seen in the uncompromised liver, whereas pronounced contribution has been reported in pathological situations (Lagasse et al., 2000). This could imply that stem cell plasticity is a rare phenomena and may reach appreciable levels only if these cells have a competitive advantage.

Another situation of human pathology where stem cell plasticity may be implied is in metaplasia, i.e., when there is a change of cell type in a certain location as a secondary effect to a pathological process (Slack and Tosh, 2001). Common examples of metaplasia include pancreas cells in the intestine, gastric epithelium in the duodenum, and endometrium in the ovary. Metaplasias are thought to be polyclonal and a way for local stem or progenitor cells to adapt to a changed environment by producing cells appropriate for the new condition.

Is there any evidence for stem cell plasticity during normal development? Although most cells are thought to progress in their differentiation along stereotype pedigrees, nature offers some quirks. For example, ectodermal neural crest cells give rise to what we in other situations consider mesodermal derivatives such as muscle, connective tissue, cartilage, and bone. Another example is epidermal placodes, thickenings of the primitive skin, which by induction from underlying structures form neural tissue. In both the case of neural crest and placode cells, the generation of not classically lineage-related progeny is instructed by the environment. Both are examples that may be viewed as switching lineages, in the case of neural crest from ectodermal to mesodermal and in the case of placodes from epidermal to neural, and may be interpreted as stem cell plasticity.

Fact or Fusion?

Cellular fusion results in a situation similar to that created in nuclear transfer experiments, where the nucleus of a cell fusing with another will be influenced by the epigenetic signals in the cytoplasm of the new partner. This can result in reprogramming to a specific lineage or a pluripotent state, depending on the cell type with which the studied cell fused.

Two recent studies suggested that at least some of the results interpreted as stem cell plasticity may be a result of cell fusion (Terada et al., 2002; Ying et al., 2002). They found that in cocultures of embryonic stem cells with brain or bone marrow cells, pluripotent hybrid cells emerged spontaneously. In neither study were stem cells from brain or bone marrow, respectively, required for this event, since the frequency was the same when comparing unfractionated brain cells with cultured neural stem cells or whole bone marrow with purified hematopoietic stem cells. Although the frequency of this event was extremely low (1:10,000–100,000 brain cells or 1:100,000–1,000,000 bone marrow cells), it stresses that adult somatic cells can gain differentiation potential by fusion with less differentiated cells (Terada et al., 2002; Ying et al., 2002), and the frequency could be higher in

other situations. Both of these studies analyzed systems conceptually different from those in which stem cell plasticity has been suggested, therefore precluding conclusions to be drawn regarding the previous studies. However, they do point out an important caveat in these types of experiments which has been overlooked and needs to be addressed in future studies.

Fusion appears to be an extremely rare event and may seem an unlikely explanation for many of the results ascribed to stem cell plasticity. On the other hand, stem cell plasticity also seems unlikely from previous knowledge. It will have to be established for each individual situation where a suspected case of plasticity is seen which of the two unlikely possibilities is true, fusion or plasticity. At this point, it is as unwise to conclude that tissue stem cells are completely plastic as to conclude that all data suggesting stem cell plasticity are due to fusion events. We need to keep our minds both open and very skeptical.

How Can Stem Cell Plasticity Be Tested?

To comprehend the possible extent of stem cell plasticity and to be able to understand the molecular underpinning, it is important to be rigorous when defining it, not least since there are ample caveats and opportunities to misinterpret findings as indicative of stem cell plasticity (Figure 2).

A first key issue is to define the cell that is studied. In many cases, heterogeneous groups of cells have been studied, which precludes conclusions regarding lineage conversion. Unless starting with a homogeneous population of cells (ideally a single prospectively identified cell), it is difficult to exclude that the starting population contained a mix of stem or progenitor cells for different lineages. For example, many studies have described the generation of various nonhematopoietic cell types such as liver and muscle cells from transplanted bone marrow. Bone marrow is, however, a complex tissue which harbors multiple different cell types and lineages, and experiments showing myocytes or hepatocytes derived from unfractionated bone marrow could in theory equally well be interpreted as presence of muscle and liver progenitor cells in bone marrow as plasticity of hematopoietic stem cells residing in bone marrow.

A related caveat may be the presence of a small number of heterologous stem cells in certain tissues. For example, cells isolated from muscle based on dye exclusion properties common to several stem cells have hematopoietic potential, and were initially interpreted as a case of stem cell plasticity where muscle stem cells could generate blood. More recently, characterization of the isolated muscle cells has suggested that the hematopoietic potential in muscle resides exclusively in a population of cells with the marker profile of hematopoietic stem cells, suggesting that such cells in the circulation may explain this result (McKinney-Freeman et al., 2002).

Yet another source of misinterpretation could be that transformed cells may generate unrelated cell lineages. The reconstitution of hematopoiesis in irradiated mice by intraveneous injection of neural stem cells (Bjornson et al., 1999) was recently suggested to be a result of transformation of neural stem cells by excessive passaging in vitro (Morshead et al., 2002).

Equally important as defining the starting point for potential plasticity is to characterize the end point, i.e., the cell type generated from the stem cell of interest. The identity of a cell is often defined by morphology and expression of appropriate markers. Ideally, one should demonstrate function of the generated cell, although this in many cases may be very difficult. This was done elegantly in one study to date, in which prospectively identified hematopoietic stem cells were found not only to generate liver cells with a marker profile expected by hepatocytes, but also to rescue mice with a genetic defect resulting in lethal liver failure (Lagasse et al., 2000). Although establishing the function of cells gener-

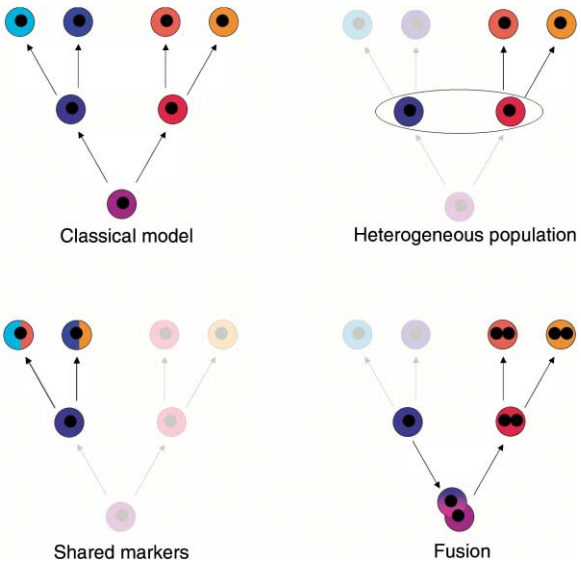

Figure 2. Situations that May Be Misinterpreted as Stem Cell Plasticity

Situations which represent differentiation of cells along a linear pedigree may easily be interpreted as suggesting stem cell plasticity. It is crucial to be certain of the starting point of the analysis, i.e., the cell type whose progeny is analyzed. Many tissues may contain heterogeneous cell populations. For example, hematopoietic stem cells are present in the circulation and in many tissues, and hematopoietic potential of cells from a certain tissue may be the result of either the plasticity of a nonhematopoietic stem cell or the presence of hematopoietic stem cells in that tissue. The analyzed cell must be characterized either by phenotype, which is well established based on cell surface markers in, for example, the hematopoietic system, or by function, which is readily done in vitro in, for example, the case of neural stem cells.

There are few cellular markers which are truly specific for a certain cell type, and it is thus dangerous to conclude stem cell plasticity based on the expression of a few markers. Moreover, a cell may phagocytize a cell of another lineage and in that way, at least transiently, acquire molecular markers of a different lineage. The unreliability of molecular markers in firmly establishing the identity of a certain cell underscores the need for evidence of function appropriate for the particular cell type. This is in many cases best shown by experiments where a certain cell type can rescue the phenotype of a mutant lacking a certain cell type or function.

Finally, cell fusion may result in hybrid tetraploid cells which will carry genetic markers of both cells. A cell which fuses with another cell may be reprogrammed by factors in the cytoplasm of the new partner, and take on properties of this cell. For example, fusion of neural and bone marrow cells with embryonic stem cells occurs spontaneously at a very low frequency in vitro, and results in pluripotent cells which can generate a variety of cell types to which these somatic cells do not normally give rise.

ated by potential stem cell plasticity will be very important and eventually necessary, it is probably overzealous and counterproductive to demand establishment of function in each individual study, since this in some cases is excruciatingly difficult. For example, although adult neurogenesis has been intensively studied the last decade, it was only recently established that adult-born neurons are functional (Carlén et al., 2002; van Praag et al., 2002).

Finally, as discussed above, cellular fusion may be misinterpreted as stem cell plasticity. An abnormal karyotype may be easy to detect, but tetraploid cells may expel supernumerary chromosomes and approach a diploid DNA complement. Analysis of distinct genetic markers is therefore required to conclusively establish whether a certain cell has a mixed karyotype with chromosomes from two different cells.

Conclusion

Transfer of nuclei of somatic cells to oocytes and the cloning of adult animals have in a striking way illustrated that in most cells there are no irreversible changes to the genome as cells differentiate, but rather the differentiated state is established and maintained by epigenetic signals. The concept of stem cell plasticity implies that cells may not only be reprogrammed in such extreme situations in which the intracellular milieu is switched by nuclear transfer or cell fusion, but that extracellular signals can reprogram cells to switch lineages. However, the fact is that none of the studies suggesting plasticity of adult stem cells have excluded all alternative explanations. Thus, it may be wise to await further studies before we revise our view of how cellular differentiation is regulated and rewrite the textbooks.

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